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Review article

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Mechanisms of assembly and remodelling of the extracellular matrix

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Abstract

The extracellular matrix (ECM) is the complex meshwork of proteins and glycans that forms the scaffold that surrounds and supports cells. It exerts key roles in all aspects of metazoan physiology, from conferring physical and mechanical properties on tissues and organs to modulating cellular processes such as proliferation, differentiation and migration. Understanding the mechanisms that orchestrate the assembly of the ECM scaffold is thus crucial to understand ECM functions in health and disease. This Review discusses novel insights into the compositional diversity of matrisome components and the mechanisms that lead to tissue-specific assemblies and architectures tailored to support specific functions. The Review then highlights recently discovered mechanisms, including post-translational modifications and metabolic pathways such as amino acid availability and the circadian clock, that modulate ECM secretion, assembly and remodelling in homeostasis and human diseases. Last, the Review explores the potential of 'matritherapies', that is, strategies to normalize ECM composition and architecture to achieve a therapeutic benefit.

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Introduction

The extracellular matrix (ECM) is the complex meshwork of proteins and glycosaminoglycans (GAGs) that forms the scaffold that surrounds and supports cells[1,2]. Phylogenetically, the build-up of the ECM coincided with the emergence of multicellularity. The diversification of the repertoire of ECM components and the complexification of ECM protein structures have accompanied crucial steps in metazoan evolution, such as the appearance of chordates, vertebrates and mammals[3–13].

The ECM exists in two structurally distinct forms, basement membranes, which are thin, sheet-like assemblies, and meshwork-like interstitial ECMs, which differ in molecular composition, architecture, localization and functions. The core of the ECM scaffold is composed of structural proteins that include collagens[14–16], fibronectin[17], fibrillins and elastin[18–23]. The ECM scaffold contributes to the organization of cells into tissues, guides cell polarization and confers mechanical properties on tissues and organs. In addition to its structural roles, the ECM both provides and relays mechanical and biochemical signals[24–28]. These signals are transduced by cell surface receptors, such as the integrins[29–32] or the discoidin domain receptors (DDRs)[33], and they modulate a plethora of cellular processes, including adhesion[34,35], migration[36–38], proliferation[39], survival[40,41] and differentiation[42]. These processes are all crucial for the proper development and homeostasis of multicellular organisms[43–45]. Consequently, disruption of the ECM structure or its signalling properties — resulting from ECM gene variants or altered ECM composition — leads to a broad spectrum of clinical presentations, from impaired development and tissue repair to cardiovascular and musculoskeletal diseases to fibrosis and cancer[42,46–50].

The mechanisms involved in the assembly and maintenance of the ECM scaffold have been primarily studied in vitro and on a small subset of exemplary proteins such as collagens[14–16,51], fibronectin[17,52], fibrillins[53,54] and elastin[18,21,55]. The study of genetic disorders of the ECM, accelerated by the sequencing of the human genome and the development of knockout mouse models, has greatly contributed to our understanding of the mechanisms of ECM assembly of a few dozen ECM components[56–60]. However, we now know that the human 'matrisome' comprises nearly 300 genes encoding structural components of the ECM and approximately 700 genes encoding proteins capable of modulating ECM protein structures and/or functions[61]. In addition, advances in proteomic technologies have revealed that the composition of the ECM is tissue specific and that any given tissue is composed of well over 200 distinct matrisome components[62,63]. Yet, the consequences of this diversity on the architecture and functions of the ECM remain to be fully elucidated.

The aim of this Review is to present recent insights into the tightly regulated and highly dynamic multistep process that leads to the assembly of the ECM scaffold. With examples primarily taken from research on the mammalian ECM, this Review discusses the compositional diversity of ECM building blocks and the mechanisms that lead to tissue-specific assemblies that result in different architectures tailored to support different functions. The Review then highlights recently discovered mechanisms that modulate ECM protein secretion, assembly and remodelling, including post-translational modifications (PTMs), and the impact of metabolic pathways such as amino acid availability, endocytic recycling and the circadian clock. Last, the Review explores the potential of 'matritherapies', that is, therapeutic approaches targeting the mechanisms of ECM assembly or cell–ECM interactions, to either maintain or restore ECM homeostasis and achieve therapeutic benefits.

Compositional diversity of the ECM

The sequencing of the genomes of various organisms and advances in our understanding of structural features of proteins have provided opportunities to perform de novo sequence analysis to identify genes encoding proteins that share common features. This approach has been used to predict many protein repertoires, including the ensemble of genes encoding protein kinases termed the kinome[64], the ensemble of G protein-coupled receptors[65] or the adhesome, that is, the complement of genes involved in cell adhesion[66].

Using characteristic features of known ECM proteins, namely, the presence of a signal peptide marking ECM proteins for secretion and the presence of protein domains commonly found in ECM proteins (for example, type III fibronectin domain, triple-helix collagen domain, epidermal growth factor (EGF)-like domain)[67] (Fig. 1), we predicted that about 1,000 genes in the human genome encode potential ECM components. We termed this ensemble the matrisome[1,61,68]. This section provides an overview of the various categories of matrisome components.

The core matrisome

The core matrisome comprises approximately 300 genes that encode proteins contributing to the structure of the ECM scaffold[61]. Based on biochemical features, these genes can be grouped into three categories: those encoding collagens, those encoding proteoglycans and those encoding other ECM glycoproteins (Fig. 1a).

Collagens. In the human genome, 44 genes encode distinct collagen chains that selectively assemble to form 28 different trimeric collagen proteins[14,51,69]. Fibrillar collagens are one of the most, if not the most, abundant proteins in mammals. Their main structural function is to confer resistance to tensile force or stretching. Collagens are characterized by the presence of triple-helix motifs composed of glycine (Gly)-X-Y repeats where the amino acids X and Y are commonly proline (Pro) and hydroxyproline (Hyp) residues[14] (Fig. 1b, left). As a result, the amino acid composition of collagens is biased towards proline and glycine residues. For example, interrogation of the protein database UniProt reveals that the α1 chain of collagen I (encoded by the gene *COL1A1*) is composed of 1,464 amino acids, of which 391 (26.7%) are glycine residues and 278 (19%) are proline residues. In comparison, glycine residues and proline residues represent 7% and 5%, respectively, of the amino acid composition of non-matrisome proteins.

Collagen chains, encoded by distinct collagen genes, form homo- or heterotrimers that further assemble into various higher-order structures allowing their classification into six groups: fibril-forming collagens (I, II, III, V, XI, XXIV and XXVII), fibril-associated collagens with interrupted triple helices (FACITs; IX, XII, XIV, XVI, XIX, XX, XXI and XXII), network-forming collagens (IV, VIII and X), transmembrane collagens (XIII, XVII, XXIII and XXV), multiplexins (XV and XVIII) and a group of four collagens characterized by the presence of multiple von Willebrand factor A (VWA) domains (collagen VI forming microfibrils, collagen VII forming anchoring fibrils, and collagens XXVI and XXVIII)[14] (representative members of these groups are presented in Fig. 1b and details of their supramolecular assemblies are discussed below). Whereas certain collagens are encoded by only a single gene, resulting in homotrimeric proteins (for example, the *COL18A1* gene encodes the α1 chain of collagen XVIII, and the functional collagen XVIII is a homotrimer composed of three α1 chains), others result from the assembly of different chains encoded by distinct genes. For example, six collagen IV genes, *COL4A1* and *COL4A2* localized on chromosome 13, *COL4A3* and

COL4A4 localized on chromosome 2 and *COL4A5* and *COL4A6* localized on chromosome X, have emerged from gene duplication. These genes are expressed in a tissue-specific manner and encode six distinct α-chains (1–6) that selectively assemble to form three functional heterotrimeric type IV collagens: a form composed of two α1 chains and one α2 chain ($\alpha1(IV)_2\alpha2(IV)$), a form composed of one α3 chain, one α4 chain and one α5 chain (α3(IV)α4(IV)α5(IV)) and a form composed of two α5 chains and one α6 chain ($\alpha5(IV)_2\alpha6$)[70]. This exemplifies the different mechanisms that contribute to the compositional diversity of the ECM.

Of note, although, arguably, the presence of a triple-helix motif is the defining feature of a collagen, not all proteins containing triple-helix motifs are classified as collagens. Interrogation of the human reference proteome in the UniProt database (UP000005640)[71] revealed that 78 genes encode proteins that contain a triple-helix motif (InterPro IPR008160)[72]. Of the 34 non-collagen genes, some, such as *EMILIN1* or the EMI domain-containing protein 1-encoding *EMID1*, encode bona fide ECM components, whereas proteins encoded by others, such as ficolins, or collectins known as soluble defence collagens[73], have been classified as ECM affiliated (see below). Yet other proteins containing triple-helix motifs, such as the macrophage receptor MARCO or some members of the scavenger receptor class A, are cell surface receptors[1,14]. Interestingly, despite several decades of active research, criteria to definitively classify a protein as a collagen remain to be defined[74].

Glycosaminoglycans and proteoglycans. GAGs are polysaccharides composed of anionic disaccharide repeats comprising one amino sugar and one uronic sugar molecule (or a galactose molecule in keratan sulfate). Hyaluronan is a GAG polymer exclusively composed of non-sulfated monosaccharides that forms a hydrated gel that fills most of the extracellular space[75]. By contrast, proteoglycans are composed of a core protein decorated by at least one covalently bound sulfated GAG chain[76,77]. The nature and position of the disaccharides, linkage and sulfation levels define four classes of GAG: chondroitin sulfate, dermatan sulfate, keratan sulfate and heparin or heparan sulfate. The primary structural function of the hydrogel formed by GAGs and proteoglycans is to resist compressive forces[78].

Proteoglycans can be classified based on the size of their core proteins into small proteoglycans of tens of kilodaltons (for example, biglycan, decorin) and large proteoglycans of several hundred kilodaltons (for example, aggrecan, perlecan, versican) (Fig. 1c). A nomenclature of proteoglycans that integrates information on cellular localization and structure has been proposed and categorizes proteoglycans into 4 major classes: a single intracellular proteoglycan, serglycin, located in secretory granules of mast cells; 13 cell surface proteoglycans; 4 pericellular proteoglycans; and 25 extracellular proteoglycans[79]. Extracellular proteoglycans are further divided into three categories: hyaluronan- and lectin-binding proteoglycans (hyalectans) and lecticans; small leucine-rich proteoglycans; and the testicans (1–3) encoded by the secreted protein acidic and rich in cysteine (SPARC)/osteonectin, CWCV and Kazal-like domains proteoglycan (*SPOCK1*–*SPOCK3*) family of genes[79]. All extracellular and pericellular proteoglycans are part of the matrisome nomenclature[61] but some differences between classifications are of note. Collagens XV (gene *COL15A1*) and XVIII (*COL18A1*) are classified as pericellular proteoglycans but are listed as collagens in the matrisome nomenclature. In addition, agrin (*AGRN*), listed as a pericellular proteoglycan, and tsukushin (*TSKU*), listed as a non-canonical small leucine-rich proteoglycan, are classified as ECM glycoproteins under the matrisome nomenclature. The matrisome nomenclature lists an additional 11 genes encoding putative proteoglycans or proteins that form complexes with proteoglycans (for example, members of the hyaluronan and proteoglycan link protein family)[61]. This highlights the difficulty of categorizing structurally and functionally multifaceted proteins and exemplifies how using different properties and parameters will result in somewhat different classifications.

Non-collagenous ECM glycoproteins. In addition to collagens and proteoglycans, nearly 200 other glycoproteins can be found in the ECM[1,61] (Fig. 1d). This number was initially predicted from sequence analysis[61] and has now found experimental support from large-scale ECM-focused proteomic studies[63] (see below). In contrast to proteoglycans that include GAG chains, the glycan moiety covalently linked to a glycoprotein can be a mono-, di-, oligo- or polysaccharide. Many of these glycoproteins, including fibronectin[17], fibrillins[22], latent transforming growth factor-β (TGFβ)-binding proteins (LTBP1–LTBP4)[58], form fibrils that contribute to the architecture of the ECM scaffold. Of the non-fibrillar components of the ECM, elastin is an amorphous protein that forms, in combination with microfibril-associated proteins, elastic fibres[20,23]. Laminins are characteristic of a specialized type of ECM called the basement membrane[80] (see below). Eleven genes in the human genome encode laminin chains (five α-chains, four β-chains and three γ-chains) that assemble selectively to form, as of today's knowledge, 16 distinct functional αβγ trimers. These assemblies are tissue and cell specific[81]. Last, matricellular proteins are non-fibrillar glycoproteins that incorporate into the ECM scaffold and exert modulatory roles within the ECM[82–84]. Examples of matricellular proteins include CCN proteins (CYR61, CTGF, NOV and the Wnt-induced secreted proteins WISP1–WISP3)[85], tenascins (TNC, TNR, TNN also referred to as TNW, TNX)[86], thrombospondins (1–5)[87] and fibulins (1–7)[88] in addition to individual proteins such as periostin[89] or SPARC[90].

ECM-associated proteins

In addition to structural ECM proteins, a plethora of proteins associate with core matrisome components and modulate the structure and/or the mechanical and signalling properties of the ECM. This subset of ECM-associated components, also defined by de novo sequence analysis and the presence of canonical protein domains, is further subdivided into 171 genes encoding ECM-affiliated proteins, 238 genes encoding ECM regulators and 344 genes encoding secreted factors[61] (Fig. 2a). Categorizing these components as part of the matrisome allowed the definition of a broad, yet comprehensive, framework for system-level studies of the ECM (see below).

ECM-affiliated proteins. As illustrated for collagens and proteoglycans, defining proteins strictly based on sequence features is challenging. For this reason, we proposed to group proteins that share structural or functional features with bona fide core ECM components but are not yet broadly accepted as ECM proteins under the umbrella term 'ECM-affiliated proteins'[61] (Fig. 2b). Examples of ECM-affiliated proteins include triple-helix-motif-containing proteins such as ficolins or collectins, galectins, mucins and surfactant proteins. Several families of transmembrane proteins were also included, for example, the proteoglycans syndecans and glypicans, semaphorins and plexins. This was motivated by experimental evidence having shown that the extracellular moiety of some proteins of these families could be released and found in association with the ECM[61].

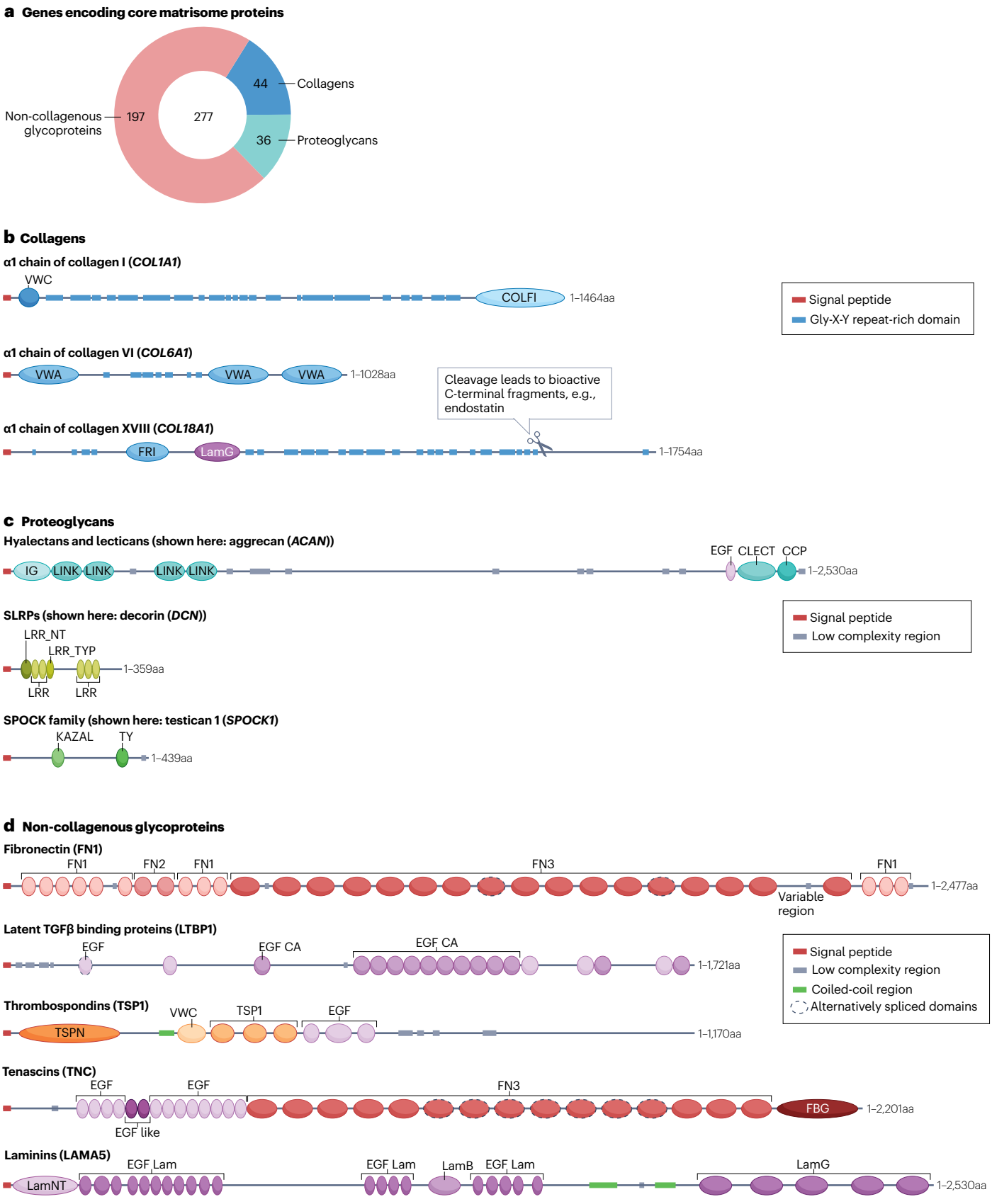

a Genes encoding core matrisome proteins
Non-collagenous glycoproteins
197
277
44
Collagens
36
Proteoglycans
b Collagens
α1 chain of collagen I (COL1A1)
VWC
COLFI
1–1464aa
α1 chain of collagen VI (COL6A1)
VWA
VWA
VWA
1–1028aa
Cleavage leads to bioactive C-terminal fragments, e.g., endostatin
α1 chain of collagen XVIII (COL18A1)
FRI
LamG
1–1754aa
Signal peptide
Gly-X-Y repeat-rich domain
c Proteoglycans
Hyalectans and lecticans (shown here: aggrecan (ACAN))
IG
LINK
LINK
LINK
LINK
EGF
CLECT
CCP
1–2,530aa
SLRPs (shown here: decorin (DCN))
LRR_NT
LRR_TYP
LRR
LRR
1–359aa
SPOCK family (shown here: testican 1 (SPOCK1))
KAZAL
TY
1–439aa
Signal peptide
Low complexity region
d Non-collagenous glycoproteins
Fibronectin (FN1)
FN1
FN2
FN1
FN3
FN1
Variable region
1–2,477aa
Latent TGFβ binding proteins (LTBP1)
EGF
EGF CA
EGF CA
1–1,721aa
Thrombospondins (TSP1)
TSPN
VWC
TSP1
EGF
1–1,170aa
Tenascins (TNC)
EGF
EGF
FN3
FBG
EGF like
1–2,201aa
Laminins (LAMA5)
LamNT
EGF Lam
EGF Lam
LamB
EGF Lam
LamG
1–2,530aa
Signal peptide
Low complexity region
Coiled-coil region
Alternatively spliced domains

Fig. 1 | Diversity of the core matrisome components. a, The human core matrisome comprises 277 genes, comprising 44 genes encoding collagens, 36 genes encoding proteoglycans and 197 genes encoding extracellular matrix (ECM) glycoproteins. The domain-based organization predicted by the simple modular architecture research tool (SMART) and members of each of these categories are depicted in the following panels. The length of the canonical form of each protein is indicated in amino acids (aa). All core matrisome components are characterized by the presence of an N-terminal signal peptide, which is required for secretion into the extracellular space. Readers are invited to visit the SMART website for more details about each protein domain depicted in the figure. **b**, The domain-based organization of exemplary collagen proteins (encoded by the *COL1A1*, *COL6A1* and *COL18A1* genes, respectively) is shown. Blue boxes indicate regions rich in triple-helix Gly-X-Y repeats (where X and Y are commonly proline and hydroxyproline residues) characteristic of collagen proteins. Other protein domains commonly found in collagens include the fibrillar collagen C-terminal domain (COLFI), the von Willebrand factor (vWF) type A domain (VWA), the frizzled domain (FRI) and the globular laminin domain (LamG). Cleavage products stemming from the C-terminal non-collagenous region of certain collagens exert biological activities, such as in the case of endostatin, an anti-angiogenic protein derived from the proteolysis of the α1 chain of collagen XVIII. **c**, Domain-based organization of extracellular proteoglycans representative of each of the three classes proposed by Iozzo and Schafer[79]: the hyaluronan- and lectin-binding proteoglycans (hyalectans) and lecticans (represented by aggrecan, encoded by *ACAN*), the small leucine-rich proteoglycans (SLRPs; represented by decorin, which is encoded by *DCN*) and the SPARC/osteonectin, CWCV and Kazal-like domain (SPOCK) proteoglycans (represented by testican 1, encoded by *SPOCK1*). Protein domains commonly found in proteoglycans include the immunoglobulin domain (IG), Link (hyaluronan-binding) domains (LINK), epidermal growth factor (EGF)-like domain, the C-type lectin or carbohydrate-recognition domain (CLECT), the complement control protein (CCP) domain (also known as short consensus repeat SCR or SUSHI repeat), various leucine-rich repeats (LRR_NT, LRR and LRR_TYP), the KAZAL domain and the thyroglobulin (TY) type I repeat. For more information on these domains, readers can refer to the SMART database mentioned above. **d**, Domain-based organization of exemplary non-collagenous ECM glycoproteins such as fibronectin, the latent transforming growth factor-β (TGFβ) binding protein 1 (LTBP1), thrombospondin 1 (TSP1), tenascin C and laminin α5. Certain matrisome genes, such as those encoding fibronectin or tenascin C, can be alternatively spliced, resulting in different isoforms containing different domains. Protein domains commonly found in ECM glycoproteins include fibronectin type I (FN1) domains that form two anti-parallel β-sheets, type II (FN2) domains that contain cysteines involved in disulfide bonds and protein–protein interactions and type III (FN3) domains that form β-sandwich folds. Another set of domains commonly found in ECM glycoproteins are EGF domains, including EGF-like domains, calcium-binding EGF (EGF CA) domains and laminin-type EGF-like (EGF Lam) domains. Other domains characteristic of ECM glycoproteins include VWC domains first identified in vWF, thrombospondin N-terminal-like (TSPN) domains and TSP1 domains first identified in thrombospondins but also found in other ECM glycoproteins, and domains first identified in laminins but broadly present in other glycoproteins such as laminin N-terminal (LamNT) domains, LamG domains and laminin B (LamB) domains. All these domains are involved in protein–protein interactions. FBG, fibrinogen-like domain.

ECM-bound secreted factors. To quote R. Hynes, one of the founders of the field of ECM research, the ECM is "not just pretty fibrils". Indeed, many ECM proteins bind to secreted factors such as growth factors, morphogens and cytokines, regulating their bioavailability and modulating their signalling functions[91]. A prime example of the interplay between ECM fibres and growth factors is that of TGFβ, which is secreted in an inactive form as part of a complex with fibril-forming LTBP proteins and whose activation depends on proteolytic cleavage and mechanical stretching[92]. The interplay between ECM and growth factors can also be exemplified by the different isoforms of vascular endothelial growth factor A (VEGFA) that are generated via alternative splicing and have different affinities for different GAGs (heparin or heparan sulfate proteoglycan) and as a result exert different signalling functions[93] (Fig. 2c).

ECM regulators. Genes grouped under the category of ECM regulators encode enzymes whose main function is to modify the physical and chemical properties of ECM components (Fig. 2d). Examples of ECM regulators include several families of proteases that cleave ECM components such as metalloproteinases of the matrix metalloproteinase (MMP), a disintegrin and metalloproteinase (ADAM) and ADAM with thrombospondin 1 motif (ADAMTS)[94], pappalysin (PAPPA) and meprin families, cathepsins[95] and elastases[96]. This category also includes proteins whose function is to modulate the enzymatic activity of ECM proteases, such as tissue inhibitors of metalloproteinases (TIMPs)[97,98], cystatins and serpin peptidase inhibitors (SERPINs). For further information on these proteolytic enzymes and regulators, readers are invited to explore the MEROPS peptidase database[99]. The ECM regulators also comprise enzymes that induce other types of PTM, such as the cross-linking enzymes of the lysyl oxidase (LOX) family[100], transglutaminases and peroxidasin (PXDN). The roles of these ECM regulators in contributing to ECM remodelling are discussed below.

Originally strictly limited to enzymes exerting an activity in the extracellular space, the list of ECM regulators was revised to include some intracellular enzymes, such as the procollagen–lysine 1,2-oxoglutarate 5-dioxygenases (PLODs), prolyl hydroxylase domain-containing proteins (EGLNs or PHDs), prolyl 4-hydroxylases (P4Hs) and sulfatases, based on experimental evidence that those enzymes remained tightly bound to their substrates and were detected in ECM-enriched protein samples by proteomics (see below)[101]. It is reasonable to speculate that a better understanding of the mechanisms that lead to the PTMs of ECM proteins will result in the expansion of the ECM regulator compendium to include more systematically all enzymes, including those exerting intracellular functions, that affect any aspect of ECM protein metabolism and perhaps even those involved in GAG metabolism.

Proteoforms increase the compositional diversity of the matrisome

Although sequence-based prediction revealed that approximately 1,000 genes encode the human matrisome, the number of proteoforms resulting from genomic (for example, single nucleotide polymorphisms (SNPs)), transcriptional (for example, use of alternative promoters), post-transcriptional (for example, alternative splicing) modifications and/or PTMs is at least an order of magnitude or two higher[102,103], contributing to increasing the diversity of matrisome components. The following sections describe some examples of this added layer of diversity.

Alternative splicing and the example of fibronectin. ECM protein isoforms result from the alternative splicing of several matrisome

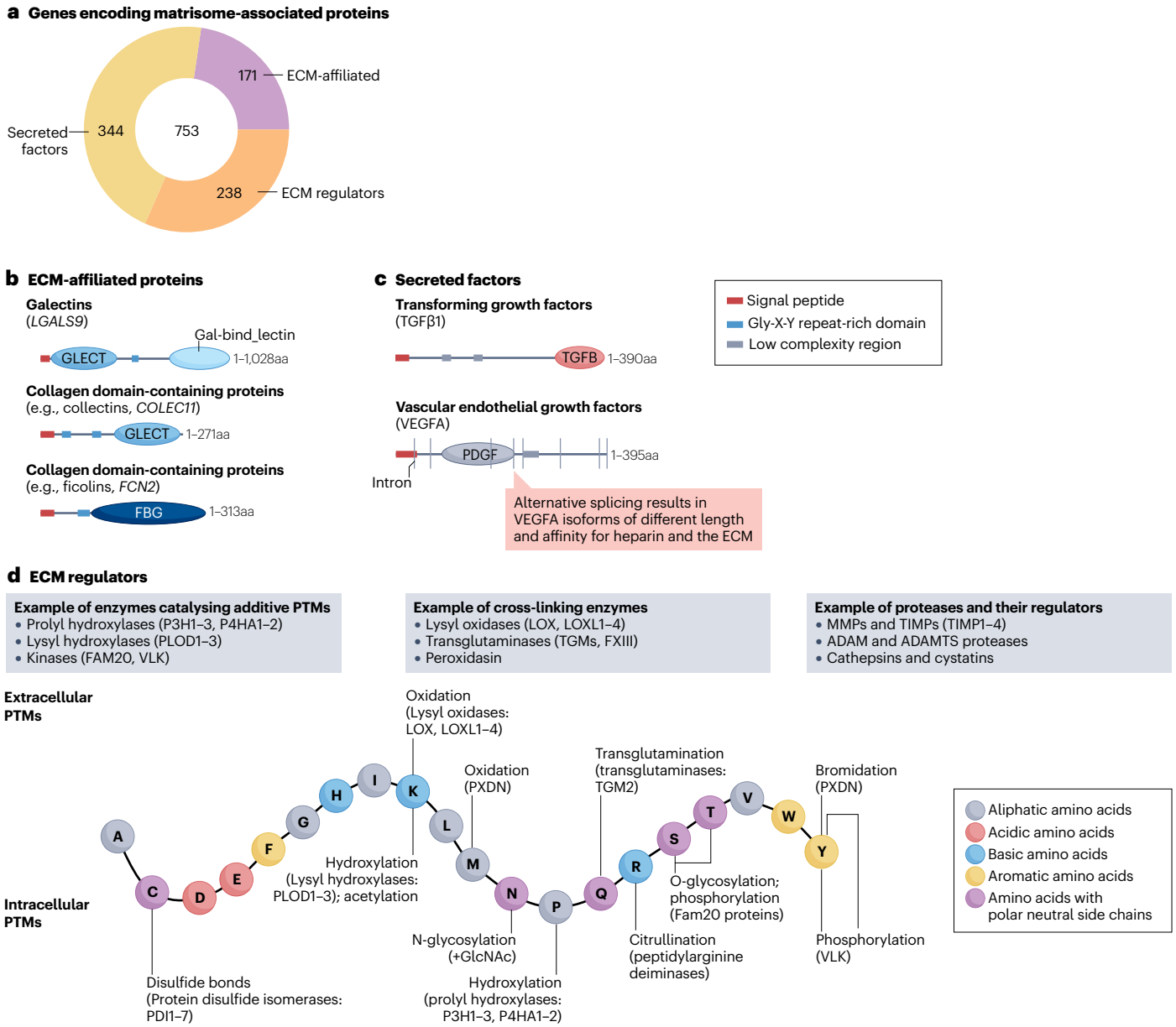


Fig. 2 | Diversity of the matrisome-associated components. a, A total of 753 genes encode matrisome-associated proteins, comprising 171 genes encoding extracellular matrix (ECM)-affiliated proteins, 238 genes encoding ECM regulators and 344 genes encoding secreted factors. The domain-based organization predicted by the simple modular architecture research tool (SMART) of canonical members of each of these categories is depicted in the following panels. The length of the canonical form of each protein is indicated in amino acids (aa). As with core matrisome components shown in Fig. 1, matrisome-associated proteins carry a signal peptide required for their extracellular secretion. **b**, Domain-based organization of exemplary ECM-affiliated proteins, including galectins (encoded by *LGAL* genes), collectins (encoded by *COLEC* genes) and ficolins (encoded by *FCN* genes). Blue boxes indicate regions rich in triple-helix Gly-X-Y (where X and Y are commonly proline and hydroxyproline) repeats characteristic of collagen proteins (see text). **c**, Domain-based organization of exemplary secreted factors known to interact with core matrisome components (for example, transforming growth factor β (TGFβ) or vascular endothelial growth factors (VEGFs)). Like core matrisome components, genes encoding matrisome-associated components such as *VEGFA* can be alternatively spliced and generate isoforms of different lengths. In the case of VEGFA, these different isoforms resulting from alternative splicing (introns are indicated by vertical bars) generate proteins that bind with different affinities to the ECM. **d**, ECM regulators can be broadly grouped into three groups: enzymes responsible for additive post-translational modifications (PTMs) (for example, prolyl hydroxylases, lysyl hydroxylases and kinases), cross-linking enzymes (for example, lysyl oxidases (LOXs), transglutaminases (TGMs), peroxidasin (PXDN)) and their regulators and proteinases (for example, matrix metalloproteinases (MMPs), a disintegrin and metalloproteinases (ADAMs), cathepsins) and their regulators. The schematic represents amino acid residues that are commonly post-translationally modified in ECM proteins and the enzymes that mediate these PTMs and whether they take place intra- or extracellularly. ADAMTS, ADAM with thrombospondin 1 motif; FBG, fibrinogen-like domain; PDGF, platelet-derived growth factor; PLOD, procollagen–lysine 1,2-oxoglutarate 5-dioxygenase; TIMP, tissue inhibitor of metalloproteinases; VLK, vertebrate lonesome kinase.

genes[104,105]. Perhaps the most striking example to illustrate how alternative splicing drives molecular diversity is that of fibronectin. Interrogation of the UniProt database reveals that fibronectin (entry P02751; canonical isoform P02751-15; 2,477 amino acids) exists in 17 isoforms produced by alternative splicing. Fibronectin isoforms present specific patterns of expression during development and disease and can bind to different partners and thus exert different functions[17,106]. For example, the isoforms EIIIA and EIIIB, also termed EDA and EDB, contain an additional type III fibronectin domain (13th or 8th, respectively) (Fig. 1d) and are predominantly expressed during development and in the pathological tumour or atherosclerotic vasculature[107]. Other isoforms differ in the size of the variable 'V' domain located between the 15th and 16th type III fibronectin domains (Fig. 1d). Recent studies have begun to uncover the complexity of the mechanisms that lead to the alternative splicing of fibronectin in the context of atherosclerosis and aneurysm: a study using mice lacking both EIIIA and EIIIB showed that these two domains exerted protective effects on the endothelium from haemorrhage and aneurysm[108]. Mechanistically, the expression of spliced fibronectin variants by endothelial cells was driven by crosstalk between endothelial cells, macrophages and platelets[108,109]. A follow-up study using ECM proteomics further showed that the inclusion of splice variants of fibronectin regulated the composition of the arterial ECM[109]. In particular, the absence of EIIIA- and EIIIB-containing fibronectin led to a decrease in the immobilization of circulating fibrillin 1 in the arterial ECM under low flow, which was proposed as a mechanism to explain why mice deficient in fibronectin splicing isoforms are at higher risk of intimal rupture. Isoforms of fibronectin containing the EIIIA and EIIIB domains have also been shown to be expressed specifically in the tumour-associated vasculature but not in the normal vasculature[107]. It has been proposed to exploit this specificity by developing bi-functional antibodies directed against these isoforms to target the delivery of drugs or imaging agents to tumours[110–114]. Isoforms arising from alternative splicing are not unique to fibronectin and similar examples can be provided for tenascin C (6 isoforms resulting from alternative splicing)[105,115,116], elastin (13 isoforms resulting from alternative splicing) but also many collagens and proteoglycans. In addition, ECM protein isoforms can arise from the use of alternative promoters (for example, *COL9A1*, *COL13A1*, *COL18A1*, LTBPs)[104].

Post-translational modifications. PTMs encompass a broad range of reversible or irreversible changes that affect the properties of a protein by the addition of chemical groups or proteolytic cleavage resulting, per the definition of the Proteomics Standards Initiative (PSI), "in an alteration of the measured molecular mass of a protein" (PSIMOD)[117,118]. Hence, PTMs constitute another major source of diversity of matrisome proteoforms. Major PTMs of ECM components are described here (Fig. 2d), and readers interested in additional details are invited to refer to a recent review for a comprehensive overview of the mechanisms and functions of ECM PTMs[119].

Apart from glycosylation, which is extensively reviewed elsewhere[120], hydroxylation (or oxidation) of proline and lysine residues constitute perhaps the most extensively studied PTMs as they are obligatory modifications of collagens[121]. The major 4-hydroxyprolines and minor 3-hydroxyprolines contribute, in large part, to the stability of the collagen triple helix at physiological temperatures[121,122]. Hydroxylysine residues, upon extracellular oxidative deamination, produce reactive aldehydes that can participate in the formation of intramolecular and intermolecular cross-links, a first step towards supramolecular assembly of collagen fibres (see below)[121,123,124].

Tyrosine bromidation is a PTM that occurs upon oxidation of bromide into hypobromous acid (HOBr) by the extracellular enzyme PXDN[125]. Interestingly, this enzyme, through its oxidizing potential, is also involved in the oxidation of methionine residues, which initiates the formation of covalent sulfilimine cross-links between methionine and hydroxylysine residues[126], a crucial step to stabilize basement membrane ECMs[127–129].

It has long been appreciated that ECM proteins could undergo phosphorylation[130]. However, the kinases responsible for the phosphorylation of secreted proteins, including ECM components, have only recently been discovered. The FAM20C kinase, discovered a decade ago, is responsible for the phosphorylation of serine residues of a large panel of secreted proteins, including matrisome components[131–133]. The vertebrate lonesome kinase (VLK) was discovered around the same time and is responsible for the phosphorylation of tyrosine residues of ECM proteins and exerts key roles in modulation of cell adhesion, migration and organogenesis[134–137].

In addition to hydroxylation, bromination and phosphorylation, many of the PTMs observed on intracellular proteins are also found on matrisome proteins, including ubiquitylation to direct proteins to proteasome-mediated degradation[138], acetylation[139] and citrullination (deamination of arginine residues)[140–142]. Of clinical relevance, citrullinated ECM components including tenascin C and collagen II are the targets of auto-antibodies in patients with rheumatoid arthritis[143–146], hence a better understanding of the mechanisms that lead to citrullination could lead to future therapeutic opportunities.

In contrast to these additive PTMs, ECM proteins can also be enzymatically cleaved by a plethora of ECM regulators (see above). As described below, proteolysis has key roles in ECM biology, including in the maturation of some collagens as pro-forms, the release of biologically active products referred to as matricryptins or matrikines[147] (Fig. 1b) and in the remodelling of the ECM after it has assembled.

Defining the matrisome of organs and tissues using mass spectrometry

Transcriptomic analyses have revealed that one of the key drivers contributing to the building of tissue-specific matrisomes is the tissue-specific regulation of matrisome gene expression[148,149]. However, gene- and transcript-level regulation accounts for only certain aspects of matrisome diversity. Over the past decade, mass-spectrometry-based proteomics has emerged as a robust method to decipher the compositional complexity of the ECM[62,150] and has revealed that the matrisome of any given tissue or organ is composed of well over 200 distinct proteins[63]. The aggregation of datasets from multiple proteomic studies on the ECM of human and murine tissues into the ECM proteomics database, MatrisomeDB, has provided experimental evidence for nearly 98% of the predicted matrisome components[63]. Comparison of the matrisome of different organs has further shown that, in addition to a subset of ubiquitous ECM proteins (such as collagens I, III and IV, fibronectin, fibrillins, LTBPs and perlecan) expressed in overall similar relative abundance, tissue- or disease-specific ECM proteins or proteoforms can be identified. As described elsewhere, this new knowledge can be leveraged to identify novel diagnostic or prognostic biomarkers and to develop novel therapeutic strategies[116,151–156].

Mass spectrometry has also been instrumental in the identification of ECM PTMs[118,157–159], including glycosylation sites by glycoproteomics[160–162], the nature of the glycans coupled to ECM proteins using glycomics[163–165] and even novel proteoglycans[166–168]. Mass spectrometry has also helped to map hydroxylation of proline and lysine residues[169].

For example, the substrate specificity of the prolyl 4-hydroxylases P4HA1 and P4HA2 was revealed by mass spectrometry, and P4HA1 was shown to preferentially hydroxylate proline residues preceded by a positively charged or a polar uncharged side chain amino acid, whereas P4HA2 preferentially hydroxylates proline residues preceded by a negatively charged amino acid[170]. Tissue-specific hydroxylation patterns were also revealed by mass-spectrometric analysis. For example, in the tendon, P4HA2 is primarily responsible for the hydroxylation of proline residues of Gly-Glu-Pro or Gly-Asp-Pro triple-helix collagen motifs[171]. Mass spectrometry has also allowed the identification of brominated proteins, substrates of PXDN, in the context of the renal basement membrane[172] and the healthy and fibrotic lung ECM[173]. Lastly, technologies such as degradomics and N-terminomics have contributed to the identification of ECM cleavage rules and products[174,175].

However, it is worth noting that certain regions of ECM proteins, sometimes large, still remain undetected by mass spectrometry, either because they are not accessible to proteolytic or chemical cleavage or because of chemical considerations that make them more difficult to resolve by liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS)[62]. As a result, low sequence coverage significantly hinders the comprehensive mapping of ECM PTMs and the identification of isoforms and single amino acid variants[176] in the context of disease. Hence, as a community we should shift our focus from aiming to increase protein identification to devising methods to increase sequence coverage. This effort will be further facilitated by creating custom protein databases, for example, through in-silico translation of RNA sequencing datasets that would include these proteoforms[177,178].

In summary, the ECM environment is incredibly large and diverse, and the mere number of 1,000 matrisome genes only begins to capture the compositional complexity of the ECM. Importantly, different ECM compositions assemble into different architectures, resulting in different tissue organizations that support broadly diverse physiological functions.

Diversity of ECM structures

As mentioned above, the ECM exists in two structurally distinct forms: basement membranes and interstitial ECMs that differ in molecular composition, architecture, localization, the nature of cell–ECM interactions and function (Fig. 3).

Basement membranes

Basement membranes are thin (tens to hundreds of nanometres) sheet-like assemblies. The core components of basement membranes include collagen IV, the glycoproteins laminins and nidogens and the proteoglycans agrin (encoded by *AGRN*) and perlecan (encoded by *HSPG2*)[179,180] (Fig. 3a). The now archived Matrixome Project website hosts the mouse basement membrane body map, which provides information on the distribution of these core basement membrane proteins in the mouse embryo. Beyond this 'basement membrane toolkit', proteomics experiments have revealed that dozens of other matrisome components can associate with the basement membrane, including collagens VI, VII, XV, XVIII and ECM glycoproteins such as fibulins, nephronectin and papillin. These are now described in the database basement membraneBASE[181].

Basement membranes are found in discrete locations including lining the basal pole of epithelial cells and creating the interface between epithelia and connective tissues (for example, at the epidermis–dermis interface), lining the vascular endothelium and surrounding certain cell types such as Schwann cells, adipocytes and myofibres (Fig. 3b). Basement membranes are also key components of stem cell niches (for example, satellite cells of skeletal muscle[182,183], epidermal stem cells located in the hair follicle bulge or the interfollicular epidermis[184,185]), although the specific ECM proteins capable of sustaining stemness remain to be identified.

Cell–basement membrane interactions are mediated by cell surface receptors including integrins and the dystroglycan receptor, and they establish a link with the actin and intermediate filament cytoskeletons through focal adhesions and hemidesmosomes, respectively[179]. Functionally, basement membranes contribute to supporting and stabilizing the structures they underlie, enable cell polarization and form a mechanical and physical barrier that contributes to key processes, including glomerular filtration or the formation of the blood–brain barrier[180,186]. Breach of the basement membrane is a crucial step in the early dissemination of tumour cells on their metastatic journey[187,188].

Interstitial extracellular matrices

In contrast to basement membranes, interstitial ECMs are porous meshworks that constitute the bulk of connective tissues (dermis, cartilage, bone) and fill the parenchyma of all organs. Fibroblasts are the primary cell type producing the fibrillar proteins of interstitial ECMs including collagens I, II, III, V, IX, fibronectins and the components of elastic fibres (for example, elastins, fibrillins, microfibril-associated proteins)[50] (Fig. 3c). Functionally, interstitial ECMs define the mechanical properties (rigidity, elasticity) of tissues and organs and support the functions of the mesenchymal cells they surround. Fibrotic disorders are characterized by excessive accumulation of interstitial ECMs[42,50].

Assembly of the ECM

The assembly of an ECM is a dynamic multistep process. This section highlights novel insights into the mechanisms that lead to ECM protein synthesis, secretion and assembly in the extracellular space. This section will also discuss newly identified regulators of these processes, including amino acid availability, PTMs and the circadian clock.

Metabolic requirements of ECM protein synthesis

ECM proteins are first synthesized in the lumen of the rough endoplasmic reticulum. ECM protein synthesis is energetically demanding owing to the large size of the proteins and presents unique challenges owing to their unique amino acid composition, in particular for collagens with high proline and glycine content[189–191]. An emerging focus of ECM biology is to study the metabolic pathways that contribute to ECM protein synthesis. The neutral amino acid transporter SLC38A2 was recently shown to be responsible for proline uptake by differentiating osteoblasts and to be required for the synthesis of proline-rich proteins such as the α1 chain of collagen I and osteocalcin (also known as bone γ-carboxyglutamic acid-containing protein, BGLAP) to sustain bone matrix production[192]. Proline and glycine are non-essential amino acids, meaning that they can also be synthesized by cells directly. A recent study showed that cancer-associated fibroblasts synthesize proline from glutamine through the conversion of 1-pyrroline 5-carboxylate by the pyrroline 5-carboxylate reductase (PYCR1) to increase collagen I synthesis[193]. Interestingly, mutation of *PYCR1* in humans results in cutis laxa, a connective tissue disorder characterized by excessively loose skin[194]. Along the same line, cancer-associated fibroblasts have been shown to use pyruvate carboxylase to fuel the tricarboxylic acid cycle to sustain levels of α-ketoglutarate, a precursor of glutamate that can be used to synthesize proline and sustain collagen I production[195]. Metabolomics has further demonstrated the existence of a feedback mechanism between ECM stiffness and arginine and

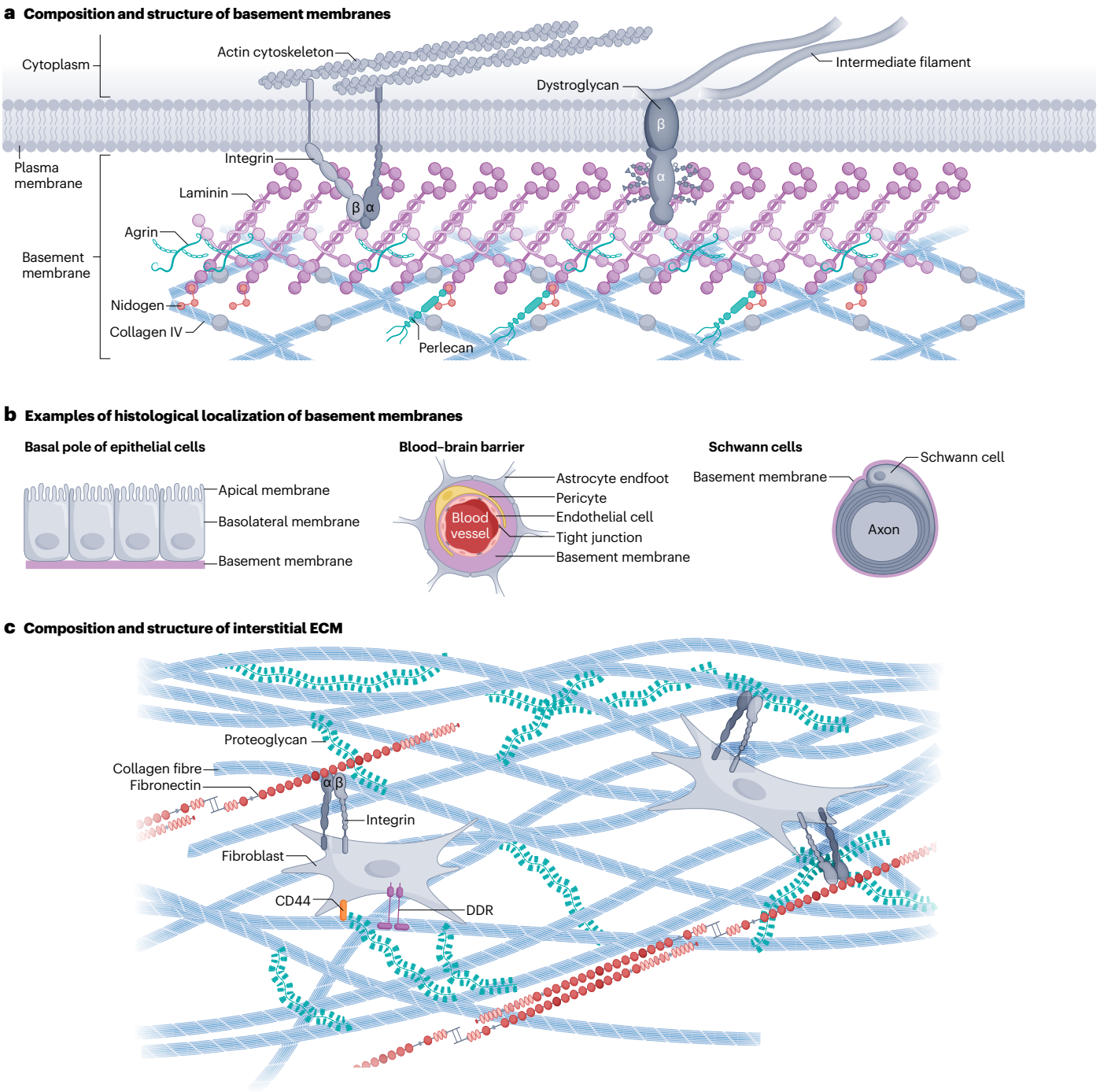
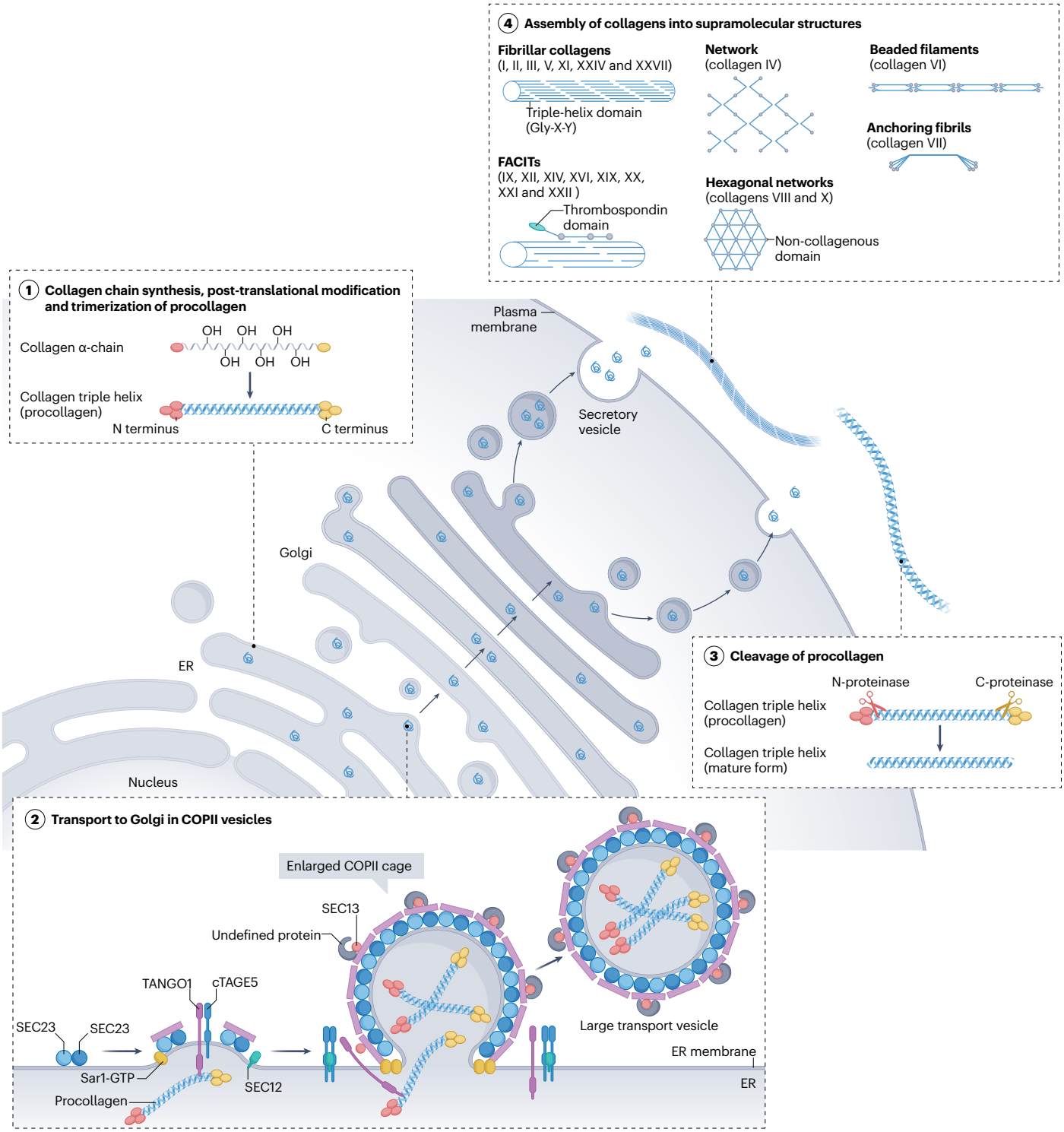


Fig. 3 | Structural diversity of extracellular matrices. a, The basement membrane is a thin sheet-like assembly of extracellular matrix (ECM) proteins primarily composed of a self-assembled lattice of laminin trimers, a network of collagen IV and the proteoglycans agrin and perlecan. Additional proteins contribute to the linkage between these components (for example, nidogen). Cells express receptors such as integrins or the laminin receptor dystroglycan at their surface ECM. These receptors create a bridge between the ECM and the cellular cytoskeleton. **b**, Examples of anatomical locations of basement membranes. Basement membranes are found in various locations, such as the basal pole of epithelial cells, surrounding endothelial cells, for example, at the blood–brain barrier interface, or around Schwann cells. **c**, The interstitial ECM is a 3D meshwork found in connective tissues and primarily made of fibrillar ECM proteins (collagens I, III, V, IX; fibronectin) and glycosaminoglycans and proteoglycans. Cells found in connective tissues (for example, fibroblasts) are surrounded by the interstitial ECM and interact with proteins of interstitial ECMs through receptors such as integrins, the tyrosine kinase discoidin domain receptors (DDRs) or the hyaluronic acid receptor CD44.



proline metabolism in murine dermal fibroblasts in vitro and showed that it was mediated by the mechanosensitive channel PIEZO1 (ref. 196), offering potential novel insights into the mechanisms of skin fibrosis. The concept of 'metabo-reciprocity' has recently emerged from this body of work to describe the bidirectional interplay between ECM mechanics and cellular metabolism[197].

ECM protein transport and secretion

In addition to being the site of protein synthesis, the endoplasmic reticulum is also the site of initial polymer assembly for collagen trimers or fibronectin dimers. The mechanisms that lead to the vesicular trafficking of ECM proteins, in particular, collagens, from the endoplasmic reticulum through the Golgi apparatus and their extracellular

Fig. 4 | Synthesis, secretion and supramolecular assembly of collagens. Extracellular matrix (ECM) proteins are synthesized in the endoplasmic reticulum (ER) and transported in coat protein complex II (COPII)-coated vesicles to the Golgi network, where additional modification steps take place. From the Golgi network, ECM proteins are transported to the extracellular space in secretory vesicles. Inset (1) depicts collagen chain synthesis and trimerization in the ER and the formation of triple-helix procollagen. Inset (2) illustrates the mechanisms of ECM cargo loading into COPII vesicles. Inset (3) shows the cleavage of collagen pro-peptides by N- and C-proteinases upon secretion into the extracellular space to form a mature collagen helix. Inset (4) depicts the diversity of supramolecular structures resulting from collagen assembly. Collagens I, II, III, V, XI, XXIV and XXVII assemble to form fibrils and higher-order fibres. Collagens IX, XII, XIV, XVI, XIX, XX, XXI and XXII are termed fibril-associated collagens with interrupted triple helices (FACITs) as they interact with fibrillar collagens. Collagens IV, VIII and X assemble to form geometrical networks ('chicken-wire-like' for collagen IV and hexagonal networks for collagens VIII and X), in which the triple-helix collagens are connected by their respective non-collagenous domains (shown in grey). Collagen VI forms beaded and collagen VII forms anchoring fibrils that bridge the basement membrane and the interstitial ECM. cTAGE5, cutaneous T cell lymphoma-associated antigen 5; TANGO1, transport and Golgi organization 1.

secretion have also been the focus of active research over the past decade (Fig. 4). We now understand that specific transmembrane cargo receptors (for example, the transport and Golgi organization 1 (TANGO1) protein, cutaneous T cell lymphoma-associated antigen 5 (cTAGE5), the KDEL receptor that recognizes the Lys-Asp-Glu-Leu amino acid sequence[198,199]), chaperones that link cargo to receptors such as heat shock protein 47 (HSP47)[200,201] and components of the coat protein complex II (COPII) (for example, members of the SEC23 and SEC24 families) are required to load large-sized ECM cargos (up to several hundred nanometres for fibrillar collagens) into COPII-coated vesicles that transport proteins from the endoplasmic reticulum to the Golgi apparatus[202,203] (Fig. 4). It has also emerged that different ECM cargos engage different partners. For example, it was recently shown that procollagen I export preferentially involves SEC24A/B, whereas fibronectin preferentially involves SEC24C/D[204]. The endoplasmic reticulum and the Golgi apparatus are also the sites of PTMs. Importantly, only adequately post-translationally modified ECM cargos, that is, those that adopt proper folding, will be trafficked on and secreted. For example, a recent study demonstrated that the secretion of collagen IV, but not of I or III, was decreased in cells lacking lysyl hydroxylase 3 (ref. 205).

Mechanisms of ECM protein assembly and ECM deposition

The building of the ECM is a multistep and multi-scale process that is mediated by protein interactions, some being initiated intracellularly and some occurring in the extracellular space. The following sections lay out the main pathways and regulatory mechanisms that lead to ECM assembly.

Self-assembly of ECM proteins. Many ECM components can spontaneously assemble. For example, collagens first assemble into triple-helix monomers in the endoplasmic reticulum (Fig. 4, inset 1) and can further assemble in supramolecular structures that allow their classification into six groups: fibril-forming collagens (I, II, III, V, XI, XXIV and XXVII), FACITs (IX, XII, XIV, XVI, XIX, XX, XXI and XXII), network- and hexagonal network-forming collagens (IV, VIII and X), beaded-filament-forming collagen VI and anchoring-fibril-forming collagen VII[14] (Fig. 4, inset 4). The self-assembly of collagens has been extensively studied for certain collagens in vitro[206]. For example, we now know that, upon secretion and cleavage of their C-terminal pro-peptides that maintain collagens otherwise soluble, fibrillar collagens can form fibrils and higher-order fibres (Figs. 1b and 4, insets 3 and 4). By contrast, triple-helix monomers of collagen VI first assemble intracellularly into anti-parallel dimers via disulfide bonds and then into tetramers. Once secreted, collagen VI tetramers further assemble into beaded filaments via interactions between their N-terminal VWA domains (Fig. 4, inset 4), a term given based on the appearance of these structures by electron microscopy[207].

Laminin trimers self-assemble into a polymeric sheet-like lattice in the basement membrane[179] (Fig. 3a). Recently, a combination of biochemical and imaging approaches has led to the mapping of the precise domains and residues involved in the trimerization of recombinant laminin chains mixed together in vitro[208,209]. Although developed based on purified proteins in vitro, the structure resulting from these recent studies has the potential to help uncover the basis of lamininopathies and the development of therapeutic strategies for these disorders[209,210].

ECM assembly relies on heterotypic protein–protein and protein–glycan interactions. Beyond the self-assembly of some ECM components, the formation of the ECM scaffold depends on heterotypic interactions involving proteins and glycans.

The example of fibronectin will be used to illustrate this (Fig. 5). Fibronectin is secreted into the extracellular space as a dimer, and its assembly into an insoluble fibrillar scaffold is a step-wise process involving, in brief: first, interaction with the cell surface receptor integrins; second, force-dependent conformational changes of fibronectin upon integrin binding and cell contractility; third, end-to-end intermolecular interactions between fibronectin dimers; and fourth, the lateral extension of fibronectin fibrils[52,211,212]. Although we now have a good understanding of the molecular requirements that govern fibronectin–integrin binding, the details of the molecular mechanisms that lead to the formation of insoluble fibronectin fibrils continue to be unravelled. For example, the early step of fibronectin assembly was recently shown to depend on the interaction of fibronectin through its 13th type III fibronectin domain with heparan sulfate[213,214] (Fig. 5b). Moreover, the cross-linking enzyme transglutaminase 2 (TGM2) was shown to bind to fibronectin and facilitate its interaction with integrins in the early step of fibronectin fibril assembly[215].

Once assembled, the fibronectin fibre scaffold serves as a substrate for the assembly of other meshworks including the collagen[216–218] and fibrillin[219,220] meshworks (Fig. 5b). The interdependence of different meshworks in the assembly of the ECM scaffold extends beyond the fibronectin meshwork. For example, the small proteoglycans decorin and biglycan can modulate the assembly and arrangement of the collagen I meshwork in the mouse tendon[221]. Similarly, the incorporation of fibrillins 1 and 2 in the ECM is dependent on its interaction with LTBP1 (ref. 222). Interestingly, the deletion of a segment of fibrillin 1 causing Weill–Marchesani syndrome impacts not only the ultrastructure of fibrillin microfibrils but also the interaction of fibrillin 1 with LTBP1, demonstrating how mutation in one ECM gene can have consequences on the entire ECM scaffold[223].

The establishment of interaction networks within the ECM and at the cell–ECM interface is of paramount importance to build the ECM

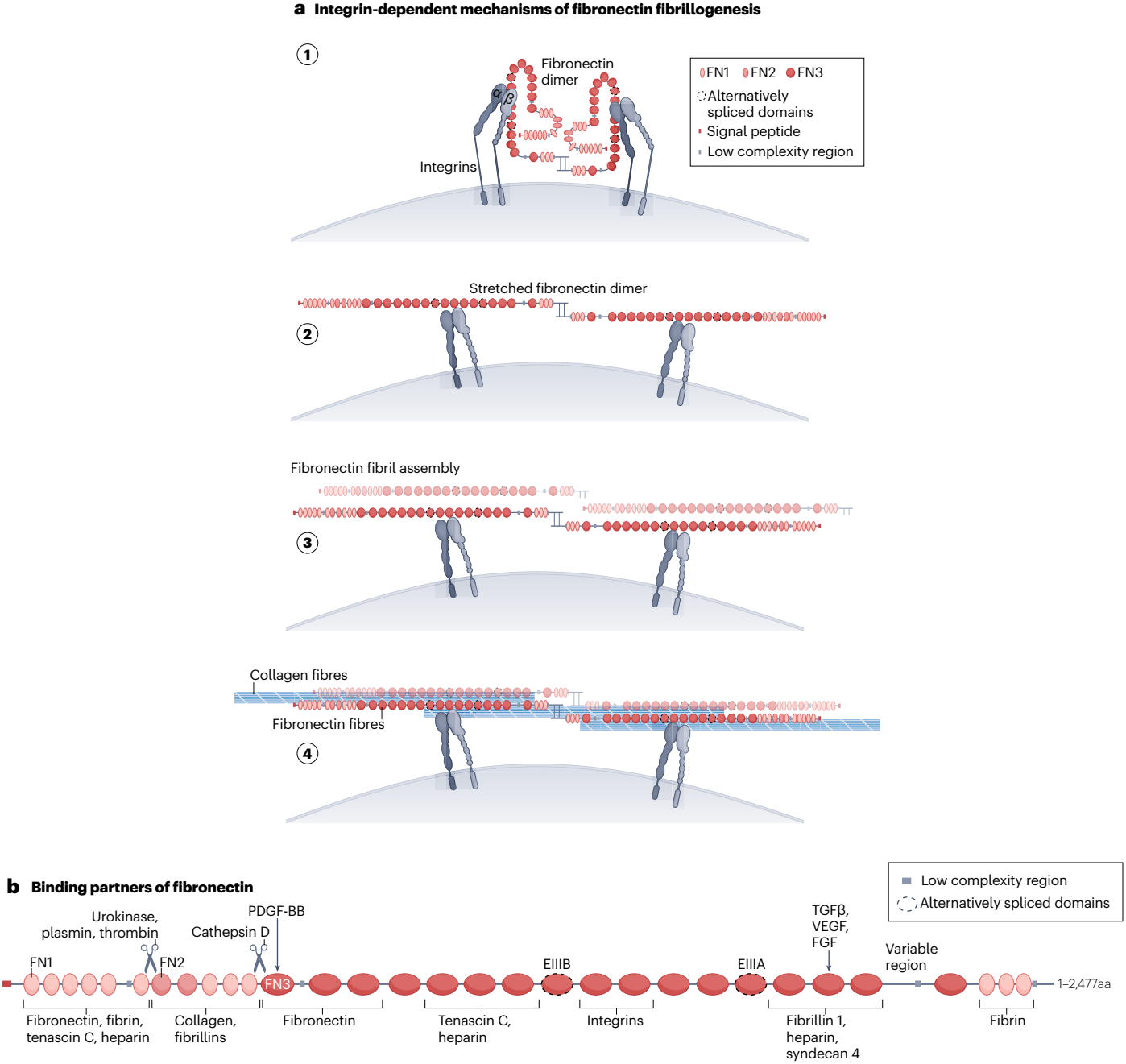


Fig. 5 | Mechanism of fibronectin fibrillogenesis. a, Fibronectin fibrillogenesis is a multistep process involving the binding of folded fibronectin dimers to heterodimeric integrin receptors expressed at the cell surface (step 1), conformational changes leading to the stretching of fibronectin dimers (step 2), the lateral extension of the fibronectin fibril array (step 3) and the assembly of collagen fibres using fibronectin fibres as a template (step 4). **b**, Map representing the domain-based organization of fibronectin and its known interacting partners and their binding sites. Notably, fibronectin interacts with cell surface receptors such as integrins primarily via its 9th and 10th fibronectin type III (FN3) domains and syndecans. It also interacts with glycosaminoglycans such as heparin (via N-terminal FN1 domains or C-terminal FN3 domains), with core matrisome proteins (for example, collagens, fibrillins and tenascin C), and with growth factors (for example, vascular endothelial growth factor (VEGF), transforming growth factor-β (TGFβ) or fibroblast growth factor (FGF)). Fibronectin can also be cleaved by proteolytic enzyme cathepsin D. A circulating form of fibronectin is found in the plasma, where it has a role in clotting by interacting with fibrin and thrombin and in haemostasis and fibrinolysis by being degraded by the proteolytic enzymes urokinase and plasmin. EIIIA and EIIIB, fibronectin isoforms; PDGF-BB, platelet-derived growth factor isoform BB.

scaffold and sustain ECM functions. Readers interested in exploring the interaction networks formed by ECM proteins can refer to MatrixDB, a database that catalogues experimentally validated interactions between intact ECM proteins or ECM protein fragments and other ECM proteins, ECM receptors, GAGs, lipids and cations[224].

Circadian regulation

In recent years, we have gained an appreciation of the highly dynamic nature of ECM assembly processes. In particular, new research has shown that the circadian clock is a key regulator of ECM biosynthesis and homeostasis. Because of its extremely well-characterized architecture and somewhat simpler composition (predominantly fibrillar collagen I), the ECM of the tendon has been the focus of investigations on what is now termed the 'chrono-matrix'[225]. In this system, the expression of several genes encoding proteins involved in collagen secretion (for example, SEC61, TANGO1) was shown to be rhythmic and to result in procollagen synthesis during the nighttime and collagen fibril assembly during the daytime[225]. Interestingly, in this system, the expression of *Col1a1* and *Col1a2* was not found to be rhythmic. The induction of collagen hydroxylation by P4HA1 in the cartilage was also recently shown to be rhythmic and to be under the control of the transcription factor and clock protein BMAL1 (ref. 226). Indeed, exposing mice to artificial light at night led to downregulation of *Bmal1* and a decrease in proline hydroxylation and collagen trafficking in chondrocytes[226]. Beyond these two examples, we now have evidence for the circadian rhythm to modulate ECM metabolism in the skin[227], cartilage[228], intervertebral discs[229] and kidney[230].

Mechanisms of physiological and pathological ECM remodelling

Once assembled, the ECM undergoes remodelling that results in new topologies (for example, bundling, alignment and pore size) and mechanical (for example, visco-elasticity and stiffness) and signalling properties. This remodelling is essential to maintain ECM homeostasis and to support cell, tissue and organ functions.

The mechanisms of physiological ECM remodelling are diverse and involve enzymatic and non-enzymatic processes, as well as cell–ECM interactions. Importantly, impairment of these mechanisms can lead to altered ECM architecture and functions.

ECM protein cross-linking

Cross-links between ECM components can be mediated by various enzymes including LOXs[100], transglutaminases such as TGM2 or Factor XIII (F13A1) and PXDN (see above). Cross-linking can also occur through non-enzymatic reactions involving advanced glycation end products (AGEs), which are proteins that become glycated as a result of exposure to glucose, fructose or derivatives[231]. Although basal cross-linking contributes to the stability and mechanical properties of the ECM meshwork and modulates physiological processes[232–235], excessive cross-linking is a hallmark of many pathological processes, including scarring[236], cancer[237] and fibrosis[49]. Interrogation of MatrisomeDB reveals that cross-linking enzymes are more abundant in the ECM of tumour microenvironments than in healthy tissues[63]. Functionally, LOX-mediated increased ECM stiffness has been shown to accompany all steps of cancer progression, from initiation to sustaining tumour cell proliferation to metastatic dissemination[238–241]. Increased collagen stiffness was shown to upregulate the expression of pro-fibrotic genes and matrisome genes in adipocytes[242]. Similarly, AGEs contribute to the initial modelling of the fibronectin and collagen meshworks[243,244]. However, excessive AGE-mediated cross-links have been shown to accompany tissue ageing[245,246], liver fibrosis[247], breast cancer metastasis[248] and clinical presentations of patients with diabetes[245,246,249,250].

ECM protein degradation and endocytosis-mediated recycling

As extensively reviewed elsewhere, proteolysis is another major mechanism that leads to the remodelling of the composition and structure of the ECM[251,252], thereby generating ECM proteoform diversity in structure and function. Proteolytic cleavage can also result in the release of bioactive molecules that exert functions different from those of native ECM proteins. For example, proteolytic cleavage of certain collagens leads to the release of anti-angiogenic peptides, including arresten, derived from the non-collagenous domain of the α1 chain of collagen IV, canstatin derived from the non-collagenous domain of the α2 chain of collagen IV or endostatin derived from the non-collagenous domain of the α1 chain of collagen XVIII[253] (Fig. 1b). Research has begun to decipher the mechanism of action of these naturally occurring bioactive fragments and has shown that they can engage ECM receptors as well as growth factors and cytokine receptors, opening the possibility of leveraging these molecules for therapeutic purposes[147,254].

Beyond the generation of biologically active fragments, we do not yet fully understand the fate of ECM cleavage products. It has now emerged that these fragments can be further catabolized or recycled. For example, proteolytic fragments of collagens can be endocytosed upon binding to the collagen receptor urokinase plasminogen activator receptor-associated protein (uPARAP or Endo180) and directed to lysosomes where they will be further digested, for example, by intracellular cathepsins[255,256]. Interestingly, the remodelling of the collagen

Glossary

Epidermolysis bullosa
Group of rare genetic skin disorders characterized clinically by skin and mucosal blistering and skin fragility.

Immune checkpoint inhibitors
Drugs that block proteins involved in immune checkpoints, the pathways that regulate activation of the immune system. In cancer imunotherapy, these drugs (for example, anti-CTLA4, anti-PD1) are used to activate antitumour immunity.

Intimal rupture
Rupture of the innermost — intimal — layer of a blood vessel wall. The tunica intima is composed of the endothelium and a subendothelial extracellular matrix including a basement membrane and elastic layer in arteries.

Liquid chromatography coupled to tandem mass spectrometry
(LC–MS/MS). Analytical proteomic method that combines peptide separation by LC and the subsequent analysis of these peptides by tandem MS.

Premature termination codon readthrough drugs
Drugs that allow the translation machinery to read through a premature stop codon and promote the synthesis of a full-length protein.

Proteoforms
Different molecular forms of a protein produced from a single gene. Can arise from single amino acid variant, alternative splicing or post-translational modification.

Schwann cells
Glial cells that form the myelin sheath of axons of the peripheral nervous system.

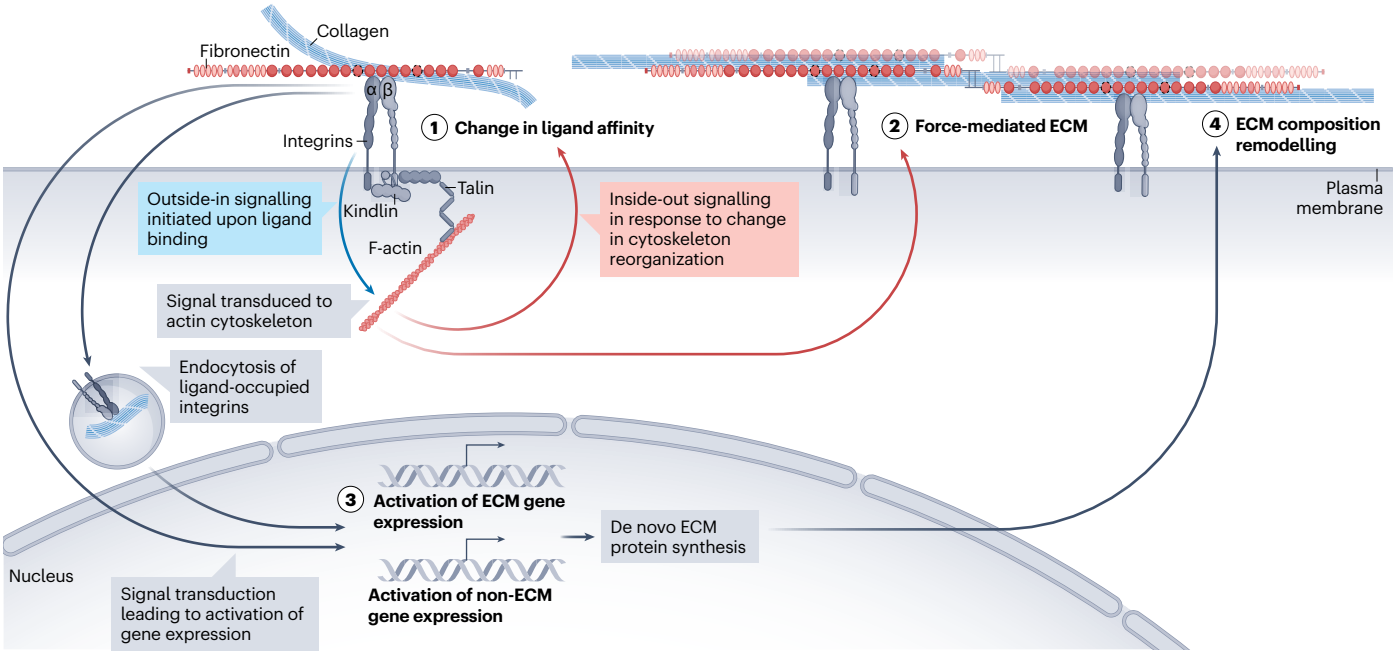


Fig. 6 | Dynamic reciprocity and mechanisms of ECM remodelling. Dynamic reciprocity describes the loop established by outside-in (blue arrows) and inside-out (red arrows) signalling between cells and the extracellular matrix (ECM). Dynamic reciprocity initiated by ECM ligand–ECM receptor interactions (for example, fibronectin–integrins) contributes to ECM remodelling in several ways: ECM ligand–integrin interaction results in a change of integrin conformation that increases the affinity of integrin for its ligand (step 1). This change in affinity results in changes in mechanical forces exerted by integrins and the cytoskeleton on the ECM ligand, but also, by proxy, on other ECM proteins binding to fibronectin (for example, fibrillins, collagens, transforming growth factor-β binding proteins) (step 2). Activation of integrin signalling — from the cell surface or from the endocytic compartment — can lead to the activation of ECM gene transcription, leading to de novo ECM protein synthesis (step 3) and consequently changes in overall ECM composition and architecture (step 4).

ECM has been shown to be under the control of the circadian clock, as expression of the collagen-degrading enzymes cathepsin K and MMP14 was shown to be rhythmic in the tendon[225,257]. Of note, collagen degradation represents an important source of proline and glycine available for collagen neo-synthesis. Similarly, the matricellular proteins thrombospondins 1 and 2 can undergo uPARAP-mediated endocytosis[258] as a way to regulate their extracellular availability. Another matricellular protein, tenascin R, characteristic of the brain ECM, contributes to ECM remodelling through cycles of endocytosis and resurfacing[259]. This constitutes a novel regulatory mechanism that can rapidly fine-tune ECM-mediated signalling.

Similarly to cross-linking enzymes, ECM-degrading enzymes, in particular MMPs and elastases, are highly abundant in the ECM of tumour microenvironments in comparison with healthy tissues[63]. These enzymes contribute to various steps of the metastatic cascade, from local invasion and intravasation to extravasation and seeding of distant organs[260]. Excessive ECM degradation also correlates with other diseases such as osteoarthritis[261], rheumatoid arthritis[261] and chronic obstructive pulmonary disease[262,263]. Although ECM protein degradation primarily impacts the architectural integrity of the ECM meshwork, it can also lead to the release of elevated levels of bioactive fragments. Consequently, the potential to use ECM degradation fragments as either biomarkers or therapeutic targets is being actively explored[264–267].

Collectively, these examples illustrate how physiological ECM remodelling results from a finely tuned balance between ECM build-up and breakdown and show how any disruption will lead to ECM disorganization and the initiation of a pathological cascade.

ECM remodelling mediated by cell–ECM interactions

As previously discussed, ECM protein interactions with their respective receptors are a crucial step in the initiation of ECM assembly. However, cell–ECM interactions, through dynamic reciprocity, are also key to remodel the ECM meshwork and create new ECM topologies in normal and pathological contexts[30,268,269] (Fig. 6). In brief, through outside-in signalling, cell–ECM interactions initiate the remodelling of the intracellular actomyosin cytoskeleton that results in changes in integrin conformation, modulating, in turn, via inside-out signalling, the affinity of integrins for their ECM ligands. Readers are invited to refer to the following references for in-depth descriptions of the mechanisms of integrin activation[28,32].

Importantly, cell–ECM interactions trigger biochemical and mechanical signals that result in structurally and compositionally different ECMs. Indeed, the forces exerted by integrins on their ligands modulate physical parameters such as ECM fibre alignment (Figs. 5 and 6). In addition, ECM-induced signalling pathways regulate the expression of matrisome genes through mechanisms that remain to be elucidated. An example of the importance of cell–ECM interactions in ECM remodelling recently emerged from a study that showed that interactions of cells with the basement membrane components collagen IV and laminin via the α2β1 and α3β1 integrins, respectively, serve as sites of initiation of fibronectin assembly. Upon initiation of fibronectin fibrillogenesis, cells can form a new type of 'sliding' α5β1-integrin-rich focal adhesion that, through interactions with the actomyosin cytoskeleton, leads to further extension of the fibronectin array[270]. Beyond signalling events that directly affect the ECM, ECM-induced signalling pathways transduced by integrins, such as the MAPK or the PI3K pathway primarily

control many cellular phenotypes including proliferation and survival[271]. Of note, recent studies have demonstrated that ECM-bound integrins could also signal from intracellular vesicles upon endocytosis[272,273].

Remodelling of the ECM during ageing

Dysregulation of ECM homeostasis in ageing exemplifies the importance of tight regulation of the mechanisms that lead to the building of the ECM. First, transcriptomic and proteomic studies have provided evidence that the expression of matrisome genes and matrisome protein biosynthesis and incorporation in the ECM vary with ageing[149,274–278]. Structural, proteomic and biophysical measurements have also highlighted that ECM proteins are altered in aged tissues through the accumulation of damaging PTMs (for example, glycation, cross-linking) that alter protein structure and consequently function[279]. Altogether, molecular damages result in the disruption of the integrity (destabilization or over-stabilization) of the ECM scaffold, with macroscopic consequences on tissues such as the skin or the cartilage. The remodelling of the ECM during ageing also creates a microenvironment that is favourable (in composition and/or structure) to the aetiology or progression of diseases that occur primarily in ageing individuals, such as age-related macular degeneration[280], prostate cancer[281] or melanoma metastasis[282].

Development of matritherapies to achieve ECM normalization

With a better understanding of the molecular make-up of the ECM, the mechanisms that lead to physiological ECM assembly and remodelling, and the mechanisms that cause altered ECM homeostasis in disease, we can now envision the development of what we propose to call matritherapies, that is, strategies to normalize ECM composition and/or architecture to restore ECM homeostasis and achieve a therapeutic benefit (Fig. 7).

Modulation of ECM gene expression, post-transcriptional and translational modifications

Targeting the signalling pathways leading to the upregulation of ECM genes (for example, TGFβ signalling pathway) has been an active route of investigation and has achieved significant success. An example of this is the discovery that the angiotensin II receptor type 1 inhibitor, losartan, could dampen TGFβ signalling in an animal model of Marfan syndrome caused by mutations in the fibrillin 1 gene *FBN1* (ref. 283) and improve the cardiovascular presentation (for example, reduction of aortic dilatation) of patients with Marfan syndrome[284]. Losartan is now being explored in the treatment of a broad spectrum of fibrotic disorders[285,286].

The possibility of modulating matrisome gene expression by intervening post-transcriptionally is also being explored. For example, gene editing and exon-skipping strategies using antisense oligonucleotides are showing promise in the context of epidermolysis bullosa caused by *COL7A1* variants[287]. A recent study reported the development of an antisense oligonucleotide targeting exon 73 of *COL7A1* in a topical gel formulation. After demonstrating that collagen VII lacking exon 73 had properties similar to that of wild-type collagen VII, the authors

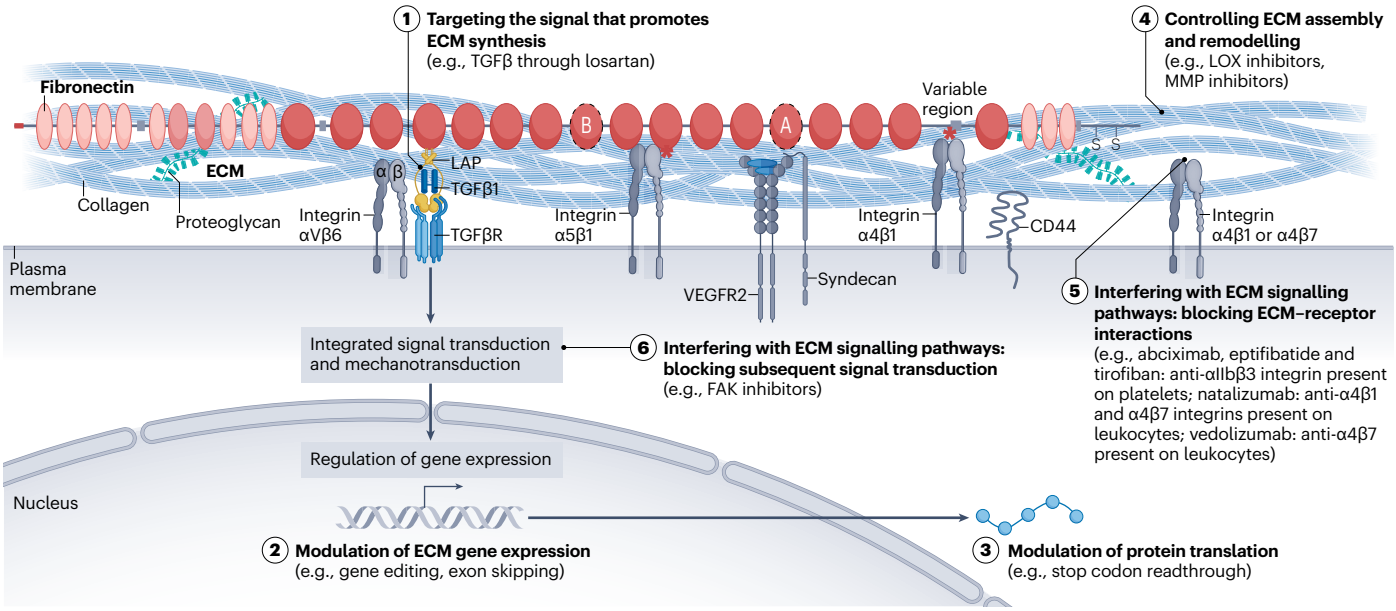


Fig. 7 | Modes of action of matritherapies. Several levels of action are envisioned to maintain or restore extracellular matrix (ECM) architecture and functions therapeutically. Modulation of ECM gene expression can be achieved by interfering with upstream signalling pathways. One such example is the angiotensin II receptor blocker, losartan, which reduces the levels of pro-fibrotic transforming growth factor-β (TGFβ) and, consequently, levels of collagens (example 1). Direct modulation of ECM gene expression and/or transcript levels can be achieved by gene editing or exon skipping, for example, by using antisense oligonucleotides (example 2). Modulation of the translation of ECM proteins can help to restore function in disease models in which mutation-induced stop codons lead to pathological protein variants. For example, drugs such as aminoglycoside and non-aminoglycoside premature termination codon readthrough drugs have been used to restore the synthesis of nonsense variants of chains of collagen IV (example 3). Modulation of the activity of ECM remodelling enzymes such as cross-linking enzymes (for example, lysyl oxidase (LOX) inhibitors) or proteases (for example, matrix metalloproteinase (MMP) inhibitors) (example 4). Blockade of the interaction between ECM ligand and its receptor (for example, integrin blocking antibodies) (example 5). Inhibition of the enzymatic activity of effectors activated downstream of ECM ligand–ECM receptor interactions (for example, inhibition of focal adhesion kinase (FAK)) (example 6). LAP, latency-associated peptide; VEGFR2, vascular endothelial growth factor receptor 2.

showed that delivery of the antisense nucleotide restored close to basal collagen VII abundance in a human skin model in vitro[288].

Last, the possibility of modulating matrisome gene expression by intervening at the translational (that is, protein synthesis) level is being researched in the context of genetic variants of matrisome genes. Nonsense variants, leading to the introduction of premature termination codons (PTCs) and the synthesis of truncated ECM proteins have been found in the *COL4A5* gene of patients with Alport syndrome[289]. An in vitro luciferase-based assay has demonstrated the potential of compounds that permit stop codon readthrough (for example, aminoglycoside and non-aminoglycoside premature termination codon readthrough drugs) to restore the expression of certain but not all variants of the α5 chain of collagen IV and further showed that the restored proteins, now including a complete C-terminal domain, can trimerize with an α3 and an α4 chain of collagen IV[290]. However, PTC readthrough-based approaches come with limitations and the risk of side effects[291] and it will be important to overcome these to allow successful clinical translation. Nonetheless, these examples demonstrate the promise of modulating ECM gene expression or ECM protein translation to achieve therapeutic benefits.

Modulation of cell–ECM interactions and ECM signalling

Modulation of cell–ECM interactions has been another active area of investigation, with a prominent focus on attempts to inhibit ECM–integrin interactions or downstream signalling effectors such as focal adhesion kinase (FAK) to disrupt ECM-triggered signalling pathways[292–294]. Successful examples include the development of biologics or small molecules to block leukocyte-specific α4β7 and α4β1 integrins for the treatment of inflammatory disorders (for example, inflammatory bowel disease, ulcerative colitis, Crohn's disease, rheumatoid arthritis) or the platelet-specific αIIbβ3 integrins for the management of thrombotic events[293] (Fig. 7). However, in these examples, the therapeutic benefit is exerted through the inhibition of integrin-dependent signalling pathways, not by achieving ECM remodelling. Nonetheless, these precedents can serve as models to devise future approaches to modulate cell-dependent remodelling of ECM architecture.

A recent example epitomizes the promise of modulating ECM signalling to achieve ECM normalization or restoration. Osteoarthritis is a debilitating degenerative disease of the joints, characterized by ECM degradation. An appealing approach to counterbalance osteoarthritis is to devise strategies to prevent ECM breakdown and/or promote ECM build-up. In a recent study, a short fragment of the secreted matrisome factor angiopoietin-like 3 (ANGPTL3) encompassing the C-terminal fibrinogen-like domain, termed LNA043, was shown to bind to α5β1 integrin and to elicit signals leading to the synthesis of cartilage ECM proteins in vitro and in vivo in a randomized phase I clinical trial. Although the mechanisms of action of LNA043 are not fully elucidated yet and its long-term benefits in vivo remain to be demonstrated, this is a promising example of matritherapy.

Modulation of the activity of ECM remodelling enzymes

Lastly, strategies that aim to modulate the action of ECM remodelling enzymes (for example, hyaluronidase, LOX, LOXL2, MMPs) to normalize ECM architecture are also envisioned. And, although early attempts ended prematurely owing to lack of therapeutic benefits or, in certain cases, severe adverse effects[295], new developments, permitted by significant advances in our understanding of the fundamental mechanisms of action of these ECM remodelling enzymes, hold tremendous potential[260,296]. For example, the LOX inhibitor β-aminopropionitrile (BAPN) has been shown to revert ECM stiffening in various tumour models, resulting in improved efficacy of immune checkpoint inhibitors[297]. However, the clinical use of BAPN has been impeded owing to its toxicity. A newly developed pan-LOX inhibitor shows promise in decreasing tumour stiffness and in improving tumour perfusion and the efficacy of chemotherapy in a mouse model of pancreatic ductal carcinoma, a tumour type characterized by its stroma content and excessive ECM accumulation[298].

Conclusions and perspectives

The past few decades of ECM research have demonstrated that the ECM is not an inert scaffold. Instead, ECMs are complex and diverse in their composition and architecture, and undergo constant, yet tightly regulated, remodelling. Many questions pertaining to ECM assembly and functions remain unanswered. For example, we have yet to build a spatially resolved atlas of the ECM at single-cell resolution to determine the positioning of ECM components with regard to each other. The ECM of a given tissue or organ is the result of a multi-scale assembly and we still do not know precisely which cell populations secrete ECM proteins, which populations secrete ECM modulators and, finally, which cell populations express receptors for these ECM proteins. Consortium-led mapping efforts, such as the NIH Human Biomolecular Atlas Program, should help to address these open questions[299]. In addition, until now, many aspects of ECM biology have been studied in isolation and in vitro, but all aspects of ECM biology are intertwined: the biochemical composition of the ECM directly affects its physical and mechanical properties and signalling functions. There is thus a crucial need to devise integrated approaches to perform system-level analyses of native ECMs together with the cells they support. Last, sustained efforts aimed at deciphering the fundamental aspects of ECM metabolism, from synthesis to secretion to assembly and post-assembly remodelling, are of paramount importance if we want to be able to devise successful matritherapies to sustain healthy ageing and benefit patients with pathologies that originate from or are aggravated by ECM disorders.

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